EFFECT OF ENZYME LOADING AND TIME ON PRODUCTION OF SUGAR FROM TAPIOCA STARCH USING ENZYMATIC HYDROLYSIS: A STATISTICAL APPROACH

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I declare that this thesis entitled "Effect of Enzyme Loading and Time on Production of Sugar from Tapioca Starch using Enzymatic Hydrolysis: A Statistical Aprroach" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree."

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Special Dedication to my family members,
my friends, my fellow colleague
and all faculty members

For all your care, support and believe in me.

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ABSTRACT

Two-step enzymatic hydrolysis of tapioca starch by commercially available α-amylase and glucoamylase was carried out in this study. Effects of enzyme loading and hydrolysis time for liquefaction and saccharification process of enzymatic hydrolysis on production of sugar from tapioca starch were investigated. A statistical approach was employed to study the effect of selected parameters where two level factorial design was applied for the experimental design. From the result, it is shown that the highest glucose concentration achieved was 277.80g/L. From the analysis of variance (ANOVA), it is found that the liquefaction time and the interaction effect between α-amylase loading and saccharification time show significant effects on the enzymatic hydrolysis. The optimum conditions suggested by the design of experiment were: 80μL α-amylase loading, 5.75mg glucoamylase loading 5.75mg, liquefaction time 1 hour and saccharification time 4 hours. From this condition, an amount of high glucose concentration was estimated.

ABSTRAK

Kaedah dua langkah proses hidrolisis enzim bagi kanji ubi kayu menggunakan enzim-enzim komersil, α-amylase dan glucoamylase telah digunakan dalam kajian ini. Kesan kandungan/amaun enzim dan masa hidrolisis bagi proses likuefaksi dan sakarafikasi dalam proses hidrolisis enzim terhadap penghasilan gula daripada kanji ubi kayu telah. Pendekatan statistik telah digunakan untuk mengkaji kesan terhadap parameter yang telah dipilih dan rekabentuk 2-level factorial. Kaedah dua tahap rekabentuk faktor telah dipilih dalam merekabentuk eksperimen. Kepekatan gula yang paling tinggi diperolehi daripada keputusan eksperimen ialah sebanyak 277.80g/L. Berdasarkan analisa varians (ANOVA), didapati masa bagi proses likuefaksi memainkan kesan yang paling ketara terhadap proses hidrolisis enzim. Selain itu, kesan gabungan kandungan enzim α-amylase dan masa proses saccharification juga memberi kesan yang signifikan terhadap penghasilan gula. Keadaan optimum yang dicadangkan daripada rekabentuk eksperimen adalah: kandungan α-amylase: 80µL, kandungan glucoamylase: 5.75mg, masa proses likuefaksi: 1 jam dan tempoh masa proses sakarafikasi: 4 jam. Daripada kondisi ini, kepekatan gula sebanyak 271.8g/L adalah dianggarkan.

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LIST OF SYMBOLS/ABBREVIATIONS

DE	-	dextrose equivalent
DNS	-	Di-Nitro Salicylic Acid
g	-	gram
h	-	hour
KNU	-	kilo novo units α-amylases
AGU	-	amount of enzyme which hydrolyses 1 mmol of maltose per minute under specified conditions
g/L	-	gram per liter
mL	-	mililiter
w/v	-	weight per volume
w/w	-	weight per weight
%	-	percentage
°C	-	degree Celsius
μL	-	microliter
CO_2	-	carbon dioxide

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

1.1.1 Oil Depletion

After the invention of steam engine in 1860's during Industrial Revolution, it has brought a bright future for humankind since then. Engine became widely applied, initially from the utilization of wood and coal, later oil and natural gas.

Fossil fuels which a successful helper for amazing economic growth were the medicine to cure deprivation. As human keep consuming the fossil fuels, two important predicaments started to emerge:

(1) the fossil fuels would be depleted in a foreseeable future since it was first being drilled in 1859.

(2) the fossil fuels is causing global environmental problem (Veziroglu *et al.*, 2008).

1.1.2 Renewable Energy

Due to the depletion of oil in oil reservoir, energy researchers are trying to find any possible alternative source of energy to replace the fossil fuels. There is numerous numbers of possible alternative primary energy sources such as thermonuclear energy, nuclear breeders, solar energy, wind energy, hydropower, geothermal energy, ocean currents, tides and waves.

Ironically to fossil fuel, none of the mentioned can be directly used as a fuel in transportation. For choosing best fuel to replace fossil fuel, the choice must satisfy the following conditions:

i. It must be convenient fuel for transportation.
ii. It must be versatile or convert with ease to other energy
iii. forms at the user end
iv. It must have high utilization efficiency
v. It must be safe to use (Veziroglu *et al*., 2008).

1.1.3 Ethanol as a Renewable Energy

A transportation sector is dependent on one finite energy source which is petroleum. Recently, ethanol has been found to be a valuable fuel, acts as alternative for the transportation sector (Wyman *et al*., 2004). Based on the statistic, most of transportation fuel comes from hydrocarbon fuel mostly in developed country. As a new transportation fuel, ethanol is favorable on resource, and environmental attributes. Ethanol is a liquid fuel that widely accepted for blending with gasoline, and can be used in core form (Wyman *et al*., 2004).

Today, there are two technically alternative methods in reducing CO_2 emissions which are increase thermal efficiency of engines and the use of liquid biorenewable fuels (Cataluña *et al*., 2008). Thus, ethanol would be the most efficient candidate.

1.1.4 Production of ethanol

Ethanol is manufactured from two major paths which are fermentation of sugars derived from sugar, starch or cellulosic material and reaction of ethylene with water. The former is favored for production of fuel. Most researchers are focusing on fermentation route since it can potentially meet fuel cost and volume need for large-scale fuel production, while ethylene is too expensive and supplies would likely be streached (Wyman *et al*., 2004).

1.1.4.1 Raw Material

In ethanol production, a critical issue has been considered is whether the process is economical. Research efforts to produce a sustainable transportation fuel are focused on design and process improvement. This can be done by using low cost feedstock in establishing a cost effective technology. In order to analyze the cost effectiveness of bioethanol, several researches had found that ethanol can be economically derived from starch (Mojovic *et al*., 2006).

High yielding ethanol resource is offered by starch. Industrial ethanol production has been reported using various starchy materials. The carbohydrates in the starchy must be pretreated in order to hydrolyze the starch to simple sugar before it is further fermented by yeasts to ethanol. Starch is hydrated and gelatinized, and particular enzyme will break down the starch polymer chain to fermentable sugar for ethanol production (Nigam *et al*., 1995).

Another potential raw material for bio-ethanol production would be lignocellulose. The principal carbohydrates contained in lignocellulose resources are the structural carbohydrates. These carbohydrates, along with proteins and lignin, form the complex matrix of plant cell walls that give plants structural stability and protection from the environment (Graf and Koehler, 2000)

Cellulose is a straight chained (linear) polymer of glucose molecules joined by ß (1-4) glycosidic bonds. The multiple hydroxyl groups (OH) situated along a cellulose chain bond with the hydroxyl groups of other cellulose chains to form tight crystalline structures (microfibrils). Microfibrils have high tensile strength and are the major structural components of all plant cell walls (Bioweb, 2008).

Compared to starch, cellulose is far more abundant in nature than is starch. However the high tensile strength and chemical stability of cellulose make it much more difficult to break down into glucose molecules. Therefore the process of converting cellulose to ethanol is more complex than that for starch. The recalcintrance feature of cellulose requires pretreatment, processing steps preceding hydrolysis of cellulose and hemicellulose into fermentable sugars. The purpose of pretreatment is to alter or remove structural and compositional factors present in plant that prevent the breakdown (hydrolysis) of cell wall polysaccharides into the fermentable simple sugars (Bioweb, 2008).

The cellulose stability make hydrolysis by using cellulose as a raw material in ethanol production become more complex than starch as an additional pretreatment process, is required. Hence, starch is a feasible raw material for bioethanol production.

1.1.5 Overview of glucose production from starch

Production of ethanol from starch required two steps which are hydrolysis of starch materials, followed by fermentation of the hydrolysis products. Hydrolysis of starch materials is a crucial step as it revealed the effectiveness of the process and raw material selected. Industrial production of starch hydrolysis products such as glucose syrup can be achieved either by using acid or enzyme catalyst. For enzymatic hydrolysis process, two enzymes basically being used are α-Amylase and glucoamylase. Conventional processes for glucose production involved liquefaction and saccharification process of starch. Liquefaction and saccharification in the process require the starch granule to be extensively gelanatinized at high temperature (Lim, 2002, Shariffa *et al.*, 2008).

1.2 Objective

The aim of this study is to obtain the optimum conditions of enzymatic hydrolysis for the production of glucose from tapioca starch. Hence the objectives of this research are:

I. To determine the effect of enzyme loading on the production of glucose from tapioca starch using enzymatic hydrolysis.

II. To determine the effect of hydrolysis time on the production of glucose from tapioca starch using enzymatic hydrolysis.

1.3 Scope of Study

The scope of the study is to determine the concentration of glucose that can be produced locally available tapioca starch via enzymatic hydrolysis. The investigation has been conducted to investigate the effects of two parameters, enzyme loading and hydrolysis time period on the glucose produced. Two-level factorial design was employed to statistically investigate the effects of enzyme loading and hydrolysis time. DNS method has been used to determine the resulted glucose from the experiment.

1.4 Problem Statement

Carbon dioxide (CO_2) gas is the main contributor for greenhouse effect. The continuation usage of fossil (hydrocarbon) fuels to meet world energy's demand is a reason for the increasing of CO_2. Almost 73 % of the CO_2 production is comes from combustion of fossil fuel. Global warming issue has made high awareness on reducing the usage of fossil fuel.

Bioethanol has been recognized as a potential alternative to hydrocarbon fuels for reduction of CO_2 emissions (Balat *et al*., 2007). Bioethanol has higher oxygen content that makes it implies less amount required additive. It also allows better oxidation of the gasoline hydrocarbon and reduce the emissions of CO_2. Greater octane booster properties make ethanol become non-toxic and do not contaminate water sources (Sánchez *et al*., 2007).

In conventional method of ethanol production, there are two processes involve which are hydrolysis and fermentation. The purpose of hydrolysis process is to breakdown raw material into while fermentation process is to convert glucose into ethanol. Acid hydrolysis and enzymatic hydrolysis is two main routes in hydrolysis process.

In acid hydrolysis, browning or charring occurred as acid is employed as catalyst. Dilute acid hydrolysis tends to produce some undesirable by-products. They are furfural and 5-dihydroxymethyl furfural, which are known to inhibit fermentation. These compound may reproduces in a small amount but they be very toxic to fermentation (Graf and Koehler, 2008). Concentrated acid hydrolysis formed fewer by-products but in order the process to be economical, the acid has to be recycled. Sulphuric acid (H_2SO_4) recycling process involving separation and reconcentration of the acid make this method becomes more complex. Neutralization of hydrolysate is required before the fermentation, which leads to sludge formation at the bottom. This is condition the required for solid–liquid separation. High temperature (150-180°C) for acid hydrolysis can degrade the sugars, reducing the carbon source and ultimately lowering the ethanol yield (Patle *et al.*, 2008).

Enzymatic hydrolysis offers major advantages over acid hydrolysis. Enzymatic hydrolysis give such as higher yields, minimal byproduct formation, low energy requirements, mild operating conditions, and low chemical disposal costs (Wyk, 2001).

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Starch

In human diet, starch is the most important energy source. Besides being an energy source, starch is widely used as a functional texturizer in food industries. Starch found naturally in granules forming most plants. It is also found in cereal seeds in maize, wheat, rice, barley and in tubers/roots from potato, tapioca) are especially rich in starch. Starch is a composition of two different types of a-glucan polymers: Generally, starch is a mixture of α-amylose (20-30%; water soluble linear polymer) and amylopectin (70-80%; water insoluble branched polymer). α-amylose, a linear molecule consisting almost exclusively of α-1, 4-linked glucose residues (Figure 2.1). While amylopectin is linear chains of α-1,4-linked glucose, also contains α-1,6-linked branch points (Figure 2.2) (Hansen *et al*., 2007).

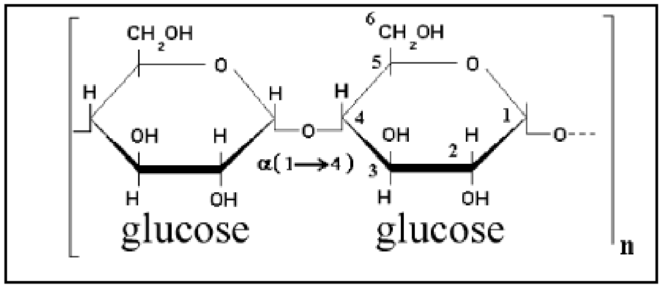

Figure 2.1: Chemical structures of α-amylase macromolecule (Reis et al,   2002)

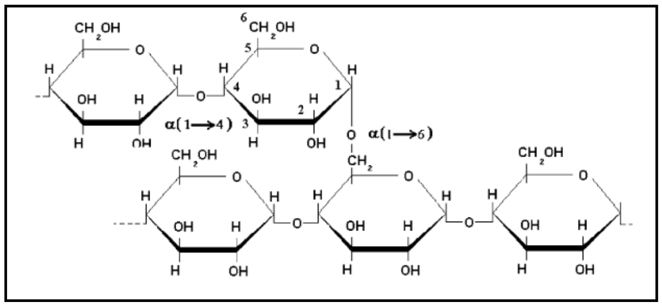

Figure 2.2: Chemical structure of amylopectin macromolecule (Reis *et al*., 2002)

The way of starch granules being stored suits to its role. Although it is insoluble in water and densely packed but it still allows for plant’s metabolic enzymes. Starch technologists who are interested in the hydrolysis of the component macromolecules noted that once the granule has been destroyed, it will be useful. Effect of hydrolysis enzyme on native starch granules has been prevailed by closer examination by many researchers (Oates, 1997).

2.2 Overview of glucose

Glucose, a monosaccharide also known as grape sugar, is an important carbohydrate in biology. The living cells use it as a source of energy and metabolic intermediate. Glucose is one of the main products of photosynthesis in both prokaryotes and eukaryotes.

From two stereoisomers of the aldohexose sugars, only one of its form which D-glucose is biologically active. This form (D-glucose) is often referred to as dextrose monohydrate, or, especially in the food industry, simply dextrose (from *dextrorotatory glucose*).

Glucose is produced commercially via enzymatic hydrolysis of starch. Many crops can be used as the source of starch. Maize, rice, wheat, potato, cassava, arrowroot, and sago are all used in various parts of the world. In the United States, cornstarch (from maize) is used almost exclusively (Wikipedia, 2009)

2.3 Hydrolysis

The carbohydrates in the starchy materials must be pretreated in order to hydrolyze the starch to simple sugar before it being pretreated by most of yeasts. Hydrolysis process for fermentable sugar involved two methods which are enzymatic and acid hydrolysis (Figure 2.3). Both two methods can be used either one. Acid hydrolysis uses acid as a catalyst for the reaction while enzymatic hydrolysis uses enzyme as its catalyst.